PROBIOTIC FORMULATION AND METHOD FOR REDUCTION OF PATHOGENIC BACTERIA

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FIELD OF THE INVENTION

The invention relates generally to a novel formulation for the control of pathogenic bacteria and more particularly to a probiotic formulation for the control of *Aeromonas*, *Pseudomonas*, *Vibrio*, *Streptococcus* and other pathogenic bacteria to fish, shellfish and other aquatic life. Furthermore, the invention relates to methods for controlling the levels of pathogenic bacteria, for promoting a healthy aquatic environment, and for promoting the health of aquatic organisms including particularly the reduction of fish morbidity and mortality. The compositions and methods are applicable in both freshwater and saltwater aquatic environments. The invention also has application for reducing pathogenic microbes in sewage or wastewater treatment facilities, specifically *Escherichia coli* or other pathogens not yet identified.

BACKGROUND OF THE INVENTION

Bacterial pathogens represent a substantial threat to aquatic environments, especially where the environment is heavily populated by fish, shellfish and other aquatic life. Aquatic environments having populations of pathogens are suboptimal for the health and development of various forms of aquatic life. Examples of such aquatic environments include a number of aquaculture type industries including, fish production facilities, where over 2.5 million pounds of fish are produced each year; freshwater and saltwater tropical fish aquariums, especially at the wholesale level where large populations of fish are combined for delivery and sale to potential customers; and koi and other outdoor ponds. Also included are intensive recirculating aquaculture systems, closed system aquaculture or other related intensive aquatic production systems containing aquatic life forms.

Prominent bacterial pathogens within the aquaculture industry include bacteria from the genus *Aeromonas*, *Vibrio*, *Pseudomonas*, *Streptococcus*, and *Columnaris*. These pathogens can lead to illness and death of the fish, shellfish or other aquatic life in the

affected environment. The cost associated with these losses, and the potential health risk to those who consume the infected aquatic life is significant, and represents a major concern within these industries. Severe infections with pathogenic microbial species can result in mortalities approaching 80-90 percent.

Presently, bacterial pathogens are controlled by widespread use of substances such as chemicals and antimicrobial agents including antibiotics. However, widespread resistance to such treatments represents a major threat to the fish and shellfish populations as well as to those who consume or are associated with the resistant bacteria. Pathogenic bacterial resistance to chemicals and antimicrobials, as well as the lack of other effective treatments is of grave concern to the aquaculture industry. Against this backdrop the present invention has been developed.

SUMMARY OF THE INVENTION

In one aspect, compositions of the present invention include the isolation of an organism or organisms useful in the reduction of other bacteria, and preferably pathogenic bacteria, in an aquatic environment. One preferred isolate, strain EHC 100 is identified as belonging to the *Bacillus cereus* species of bacteria.

In another aspect, a method for reducing the levels of a bacteria, and preferably a pathogenic bacteria, in an aquatic environment includes providing the compositions of the present invention to the aquatic environment and monitoring the levels of pathogenic bacteria in the aquatic environment.

In another aspect, compositions of the present invention are used in the prevention of fish and shellfish mortality and in the treatment of fish and shell fish having a bacterially related disease.

These and various other features as well as advantages which characterize the present invention will be apparent from a reading of the following detailed description and a review of the associated figures.

BRIEF DESCRIPTION OF THE FIGURES

Fig 1 illustrates a reduction in pathogenic bacteria levels in an aquatic environment treated with a composition in accordance with the present invention.

Fig 2 illustrates a reduction in *Tilapia* fingerling mortality in an aquatic environment treated with a composition in accordance with the present invention.

Fig 3 illustrates a reduction in pathogenic bacteria levels in a koi pond treated with a composition in accordance with the present invention.

Fig 4 illustrates a reduction in pathogenic bacteria levels in a koi pond treated with a composition in accordance with the present invention.

Fig 5 illustrates a reduction in pathogenic bacteria levels in a koi pond treated with a composition in accordance with the present invention.

Fig 6 illustrates a reduction in pathogenic bacteria levels in an aquaculture with a *Bacillus* EHC 100 strain of the present invention.

Fig 7 illustrates a reduction in vibrio pathogens in 0.8 ha shrimp ponds using the *Bacillus* EHC 100 strain of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Probiotic Organism

As described more fully in the Examples below, a probiotic organism that reduces the levels of pathogenic bacteria in aquatic environments has been identified and characterized. The identified organism belongs to the *Bacillus* genus of bacteria, and in particular, belongs to the *Bacillus cereus* species of bacteria. According to the Biolog system of classification, the probiotic organism falls into Group 3 Gram-positive spore-forming rods.

In precisely classifying the organism taxonomically, there may be instances of comparison where the identified organism appears similar to other organisms. For example, *Bacillus thuringiensis* (producer of insecticidal toxins) and *Bacillus anthracis* (etiological agent of anthrax) may be closely related to the identified organism. There is significant similarity among these organisms according to several aspects such as fatty acid analysis, and DNA sequence analysis including data regarding the intergenic space for ribosomal genes. The identified organism, however, is distinct in having the properties of significantly affecting the level of pathogenic bacteria in an aquatic environment and affecting fish health in aquaculture systems.

The probiotic organism of the present invention was identified using an activity assay, where a previously described waste and sludge reducer (K.I. Waste & Sludge Reducer, Keeton Industries, Fort Collins, CO) (hereinafter K.I. Reducer) was analyzed for its effects on *Aeromonas* and *Pseudomonas* levels in an aquatic environment. K.I. Reducer itself is used for control of accumulated organic waste and sludge in ponds, lakes, ornamental ponds, aquaria, and aquaculture facilities; also, it reduces nutrient levels through accelerated microbial decomposition of organic wastes on the pond bottom. In addition, K.I. Reducer also decreases the negative effects of eutrophication, the bio-oxygen-demand status, organic waste digestion and the rapid overgrowth of some bacteria and algae in aquatic environments.

From selected batches of the K. I. Reducer, smaller aliquots were prepared and tested for the ability to cause a decrease in pathogenic bacteria levels. Standard microbial isolation techniques and identifications using fatty acid analysis were performed on samples having the greatest effects on pathogenic bacteria reduction. Samples from a batch having the greatest activity were selected for further testing. Further testing showed that an isolated organism from K.I. Reducer showed significant activity in reducing pathogenic bacteria levels in aquatic environments. This isolate, designatedstrain EHC 100, was identified as a strain of the species *Bacillus cereus* by standard microbiological techniques and fatty acid analysis. This isolate has been deposited with the Agricultural Research Service Culture Collection (NRRL), 1815 North University Street, Peoria

Illinois, having accession number _____. Note that as used herein, "isolated" refers to an organism of the present invention that has been separated from at least one contaminant found in the organisms natural environment or found in K.I. Reducer.

Formulations of *Bacillus* EHC 100 strain were fermented and blended in solutions of 2 to 5% sodium. These blends were tested for the ability to reduce a level of pathogenic bacteria. Note that other like salts may be substituted for sodium.

The *Bacillus* EHC 100 strain compositions can be blended into other compositions that contain other species of beneficial microorgnisms. A blend can retain one or more than one property such as having the ability to reduce pathogenic bacteria in an aquatic environment and the ability to improve fish health.

Here, the terms "reduce" and "control" are used interchangeably and refer to a statistically significant difference in the level of a pathogenic bacteria in a target environment, or in the overall level of bacteria other than any composition used for treatment, when the difference relates to a level prior to treatment compared to a level following treatment.

Some conditions may affect the effectiveness of the compositions in an aquatic environment to reduce pathogenic bacteria or to improve fish health. In some cases, the effectiveness of any of the compositions can be dependent on the total alkalinity of the aquatic environment, or the temperature of the aquatic environment. For example, an aquatic environment having a temperature of about 50°F to 62°F may require an enhanced dose of the composition to produce the same effects as an aquatic environment at about 63°F to 68°F. Therefore, there is likely a sliding scale of how much of the composition is required to produce the same effects on the aquatic environment dependent on the temperature of the aquatic environment. Other conditions that may affect effectiveness of compositions include whether a pond is heavily stocked or has poor filtering. Regardless, the dose of the composition may be varied to suit the conditions of the aquatic environment and produce the expected results.

Mechanisms of Action

The mechanisms of action are currently under investigation with regard to the ability of the compositions of the present invention to affect levels of pathogenic bacteria or to affect the health of aquatic organisms. All possible mechanisms of action for the present invention are not intended to limit the scope of the invention in any way and are all believed to be within the scope of the present invention.

One potential mechanism of action is for a composition of the present invention to directly compete with other bacteria, such as pathogenic species of bacteria for resources, such as nutrients in the aquatic environment. The compositions of the present invention may prevent other bacteria from maintaining or increasing their population levels. Such direct competition is effective in cases where the organisms, for example EHC100, in the composition are present initially with greater numbers, or in a case where an organism in the composition grows at a greater rate than one or more other species of bacteria in the target aquatic environment.

A second potential mechanism involves indirect competition, where an organism in the composition fosters an environment that affects a third party organism in the aquatic environment. For example, the third party organism may then be in direct competition with the pathogenic species of bacteria in the aquatic environment.

A third potential mechanism involves the production of a molecule by an organism in the composition, for example EHC100 , where the molecule acts either directly or indirectly upon a species of pathogenic bacteria in the aquatic environment. Such molecules could be peptides that function as toxins, or enzymes.

A fourth potential mechanism involves the production of a molecule by an organism in the composition, for example ECH100, that induces pathogen resistance in the aquatic organism population (similar to Systemic Acquired Resistance (SAR) in plants).

The possibility exists that some level of each mechanism is involved, independently or in combination, to give rise to the activities of the compositions described herein. It is also anticipated that an as of yet undiscovered mechanism of action may also be involved with the mechanisms of action of the present invention.

Culture Conditions

Initial compositions were isolated as pure cultures in standard media as known in the art such as nutrient agar or trypticase soy agar. Compositions that are embodiments of the present invention are grown in liquid broth cultures. The media can be a conventional media known in the art such as trypticase soy broth (TSB). Other enrichment media may also be used to grow compositions.

At the end of culturing, the density of the target organism is preferably between 4 x 10^8 and 6 x 10^8 cfu/mL, although greater or smaller concentrations may be attained and used according to the invention. In the cultures, about at least 80% of the colony forming units can be spores. In growing the cultures, a range of about 10°C to 37°C is used, preferably a range of 20°C to 34°C is used, and most preferably, a range of 28°C to 30°C is used. The cultures are incubated for about 12 to about 72 hours, and preferably for an amount of time required to reach the preferred cell density in a given culture medium.

A completed liquid culture can be stored at refrigeration temperatures or at room temperatures. Preferably, the cultures are stored at from about 3°C to about 25°C. In another embodiment, the liquid culture may be dehydrated for storage and re-hydrated upon use. In yet another embodiment, the liquid culture may be lyophilized and stored. A culture may retain effectiveness for applications when stored for several months at room temperature and for over a year at refrigeration temperature.

Note that compositions may be used in aquatic applications in the presence of other products, for example, antibiotics which are well known in the art.

Applications

The organisms in compositions of embodiments of the invention are effective at reducing pathogenic bacteria levels in aquatic environments. In the methods of the invention, the pathogen-reducing effects of the present invention are achieved by treating an aquatic environment with one or more doses of a composition.

Dose sizes can range from about 20 mL to 60 mL of approximately 4 x 10^8 to approximately 6 x 10^8 organisms per 1,000 gallons of aquatic medium. Doses can be adjusted depending on a variety of factors including level of stocking, level of filtering, temperature, and alkalinity. Note that doses can be repeated as needed to achieve the desired result.

Other methods are envisioned to be within the scope of the present invention, including methods for treating a population of aquatic organisms such as fish or shellfish, methods for preventing bacterially transmitted disease in aquatic organisms, methods for improving an aquatic environment; methods for isolating an organism used to reduce pathogenic bacteria levels in an aquatic environment, where the desirable properties of the target organism are used to screen for the targeted organism; and the like. With respect to the present invention, "treatment" of fish or shellfish in an aquatic environment is meant to refer to any reduction in the percentage of fish or shellfish infected with a sufficient level of pathogenic bacterium that the fish or shellfish exhibit symptoms of the infection, for example, exhibit sores or blisters. A treatment, as such, is accomplished if a detectable number of fish or shellfish in the aquatic environment exhibit either fewer symptoms then before or have been alleviated of symptoms, by the compositions of the present invention being added to the environment.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1: Probiotic Solution Decreases Pathogenic Bacteria

As shown in Fig. 1, addition of a volume of about 10-20mL of product per 1,000 gallons system volume with a concentration of about $4x10^8$ cfu/mL of *Bacillus* EHC 100 strain to an aquaculture system having up to approximately 10^5 colonies per mL of *Streptococcus*, *Pseudomonas* and *Aeromonas* resulted in a dramatic decrease in the pathogens concentrations. Pathogens were substantially eliminated over the course of one month of treatment.

Similar results are shown in Table 1, where treatment of multiple strains of hemolytic streptococci with the probiotic composition reduced the level of hemolytic streptococci to undetectable levels as measured over a two-week period. Consistent results were observed a second experiment, also shown in Table 1. These experiments involved a commercial closed system Aquaculture Facility. A volume of about 10-20mL of product per 1000 gallons system volume with a concentration of about $4x10^8$ cfu/mL of the probiotic composition was used for an aquaculture environment having a volume capacity of about two million gallons. All analytical microbiological tests were performed by a certified laboratory using conventional methods as known in the art.

The data illustrates that the probiotic solutions of the invention are associated with a reduction in target pathogenic bacteria in an aquaculture environment.

Table 1 illustrates treatment with *Bacillus* EHC 100 strain in intensive aquaculture system. Beta hemolytic streptococcus was isolated in two production sytems at 2000 cfu/ml in system I and 1000 cfu/ml in system II , respectively. The systems were treated with three applications of strain EHC100 atthe recommended dosage of 10-20 ml, *Bacillus* EHC 100 strain per 1000 gallons of system volume. Two weeks after the third application , no pathogenic beta hemolytic *Streptococcus* bacteria were found in the fish tank growout system.

The aquaculture tanks were screened for *Steptococcus* organisms only, as prior testing had confirmed that fish were infected with *Streptococcus*.

Further evidence that the probiotic strain EHC 100 causes a reduction in pathogenic organisms is provided in Figures 2 and 3. Figure 2 illustrates a reduction of pathogenic organisms in outdoor shrimp ponds following treatments with *Bacillus* EHC 100 strain . Pathogenic organisms where isolated and identified as *Vibrio, Pseudomonas aeruginosa, Enterobacter* sp. And *Proteus* sp at the start of testing . Applications of *Bacillus*EHC 100 strain were added at a rate of 1 liter per 100,000 gallons of pond water, 1 treatment each 3 days for nine days followed by one weekly treatment for a 28 day period. Pathogenic organisms were decreased from 15,000 CFU/ml to 13 CFU/ml at the end of 28 day period. All tests were performed by qualified state pathologist at the Government Laboratory Of Diagnostic Veterinarial Science.

Figure 3 provides a graph that depicts reduction of *Vibrio* bacterial pathogens in a 0.8 hectare intensive culture outdoor shrimp pond in the Republic of Ecuador. *Vibrio* was completely controlled by normal applications of *Bacillus cereus*, EHC 100 strain, during the entire 120 day growout cycle for shrimp *Paneaus vannemei* (White Shrimp)

Table 1. Effect of B. cereus, EHC 100 strain on control of pathogenic bacteria

	Results	
Site	Control (cfu/mL)	Treated (cfu/mL)
Experiment I	2×10^3 2000 hemolytic streptococci/ml before treatment	< No beta hemolytic strep detected two weeks after treatment. Detection limit of 1 CFU per mL of hemolytic streptococcus
Experiment II	1×10^4 1000 hemolytic streptococci /ml before treatment	< No beta hemolytic strep detected after two weeks after treatment detection limit of 1 CFU/ml streptococcus

Example 2: Probiotic Solution Decreases Tilapia Mortality

As shown in Fig. 4, probiotic solution treatment of *Tilapia* fingerlings resulted in a significant decrease in mortality. Approximately one liter of probiotic solution having a concentration of $4x10^8$ bacillus of the invention was added per 100,000 gallons of system volume three times in 9 days followed by weekly treatments over a four week period. Fingerling mortality was monitored over a four week period.

The data illustrates that the probiotic solutions of the invention have a positive effect in reducing the mortality of treated aquatic populations.

Example 3: Probiotic Solution Decreases Pathogenic Bacteria

As shown in Figs. 5, 6, and 7, and Table 2, addition of a probiotic solution of the present invention to various aquatic systems including ponds resulted in a significant decrease in the pond water levels of certain target pathogenic bacteria.

In these examples, the concentration of bacteria of the composition in the initial 20ml dose was about $4x10^8$ CFU/mL to 6 x 10^8 CFU/mL.

Fig 5 shows the results of treating Pond A with a composition at a dose rate of 20 mL per 1,000 gallons of aquatic medium. Pond A water initially having approximately 25,000 to 30,000 *Aeromonas* and *Pseudomonas* cfu/mL was treated with a probiotic solution of the invention. A period of treatment lasting about 19 days resulted in a significant decrease in the pathogens levels: below 3,000 cfu/mL for *Aeromonas* and undetectable levels for *Pseudomonas*.

Fig 6 shows the results of treating Pond B water with a composition at a dose rate of 20 mL per 1,000 gallons of aquatic medium. Pond B water initially had levels of bacteria as follows: *Aeromonas sobria* at 10,000 cfu/mL, *Aeromonas hydrophilia* at 20,000 cfu/mL, and *Pseudomonas* at 160,000 cfu/mL. A period of treatment lasting about 32 days resulted in a significant decrease in the pathogens levels. At the end of the treatment

period, the following levels were observed: *Aeromonas sobria* was undetectable, *Aeromonas hydrophilia* at 3,000 cfu/mL, and *Pseudomonas* was undetectable.

Fig 7 shows the results of treating a koi system with a composition at a dose rate of 20 mL per 1,000 gallons of aquatic medium and at a dose rate of 60mL per 1,000 gallons. The koi system water initially had levels of bacteria as follows: *Aeromonas sobria/hydrophilia* at 500,000 cfu/mL, and *Burkholderia cepacia* (Pseudo) at an undetectable level. Several treatment periods were examined, including periods of 18, 48, and 97 days. After the initial treatment at a dose rate of 20mL per 1,000 gallons, an assessment was made at 18 days. At 18 days, the following levels were observed: *Aeromonas sobria/hydrophilia* at 500,000 cfu/mL, and *Burkholderia cepacia* (Pseudo) at an undetectable level.

After the 18 day period, the dose rate was increased to 60ml per 1,000 gallons. After 48 days, a significant change was noted in at least one population of bacteria. At 48 days, the following levels were observed: *Aeromonas sobria/hydrophilia* at 35,000 cfu/mL, and *Burkholderia cepacia* (Pseudo) at level of 60,000 cfu/mL. After 97 days, the following levels were observed: *Aeromonas sobria/hydrophilia* at 16,000 cfu/mL, and *Burkholderia cepacia* (Pseudo) at an undetectable level. Thus the treatment ultimately resulted in a reduction of more than 10-fold for *Aeromonas* species after 48 days, with a reduction of more than 25-fold after 97 days.

Treatments during the first 18 days were standard dosing of 20 ml/1000 gallon system water , one treatment every 3 days for 9 days , followed by once per week.

Some of these experiments involved an outdoor pond. All analytical microbiological tests were performed by a certified laboratory using conventional methods as known in the art.

Table 2. Data supporting Figures 5, 6, and 7.

Fig. 5	Organism	Before treatment, Day 0	After treatment, Day 19
.	Aeromonas	30,000	3,000
.	Pseudomonas	25,000	none detected

Fig. 6	Organism	Before treatment, Day 0	After treatment, Day 32
.	Aeromonas sobria	10,000	none detected
.	Aeromonas hydrophilia	20,000	3,000
.	Pseudomonas	160,000	none detected

Fig. 7	Organism	Before treatment, Day 0	After treatment, Day 18	After treatment, Day 48	After treatment, Day 97
.	Aeromonas sobria + Aeromonas hydrophilia	500,000	500,000	35,000	18,000
.	Burkholderia cepacia (Pseudo)	none detected	none detected	60,000	none detected

Example 4: EHC100 Has A Fatty Acid Composition Consistent With That of *Bacillus cereus*

The fatty acid composition of an individual species of bacteria can serve as a reliable identifying characteristic. In general, a sample of unknown bacteria is cultured and its fatty acids extracted for separation by gas chromatography. A computer generated profile of the separated fatty acids (having from 9 to 20 carbons in length) is compared to profiles for over 2600 species in a Microbial Identification System (Sherlock Libraries). Matches are determined through statistical probability software.

Fatty acid analysis on a target sample can be performed by a commercial laboratory, for example, Acculab, 223 Lake Drive, Pancader Corporate Center, Newark, Delaware. Briefly, Acculab receives a target culture, grows and harvests the bacteria, and places the bacteria in an extraction tube. One ml of a saponification solution (90g sodium hydroxide mixed with 300 ml methanol and 300 ml dH_2O) is added to the sample and the sample is heated in a boiling water bath for 5 minutes. Samples are removed from the

water bath and vortexed for about 5 seconds. The samples are returned to the water bath for an additional 25 minutes at which time it is removed and cooled in tap water. Two mls of methylation solution (405 mls of 6 N hydrochloric acid added to 345 ml of methyl alcohol) is added to the sample and the sample is heated for about 9 to 11 minutes at 79 to 81∘C. The sample is removed and cooled in tap water. Approximately 1.5 mls of extraction solution (400 ml of hexane mixed with 400 ml of methyl tert-butyl ether) is then added to the sample and the sample is gently tumbled for about 10 minutes. The aqueous lower phase is discarded and 3 mls of base wash (9g sodium hydroxide dissolved in 750 ml dH_2O) is added for an additional 5 minute incubation. Approximately 2/3 of the organic phase is removed and analyzed by gas chromatography (MIDI BIO-GC, Hewlett-Packard) using a fused silica capillary column (coated with 5% phenyl methyl silicone). GC generated data is analyzed using MIDI BIO-GC software.

Results are computed to provide a numeric value which expresses how closely the fatty acid composition of the unknown sample compares with the mean fatty acid compositions of the strains used to create the Sherlock Library. A value with a similarity above 0.5 or higher and more than 0.1 separation between the first and second entry is considered a good library comparison.

As shown in Table 3, EHC100 has a fatty acid composition that likely corresponds to the fatty acid composition of *Bacillus cereus*. A sample of EHC100 was isolated using methods in accordance with the present invention and supplied to Acculab. The EHC100 sample showed a 0.752 value, which was over 0.1 greater than the next entry - 0.619 (*Bacillus canadensis*), indicating that there is a good likelihood that EKC100 is a *Bacillus cereus*.

Table 3: Acculab Generated Profile and Similarity Index for EHC100

TABLE 3

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.597	447011437	0.028		7.014	SOLVENT PEAK		< min rt	
1.698	17350	0.016		7.222			< min rt	
1.781	7784	0.133		7.394			< min rt	
4.247	493	0.028	1.060	11.608	12:0 ISO	0.36	ECL deviates -0.001	Reference 0.006
5.279	10541	0.032	1.014	12.614	13:0 ISO	7.39	ECL deviates -0.000	Reference 0.006
5.378	1491	0 034	1.011	12.703	13:0 ANTEISO	1.04	ECL deviates -0.001	Reference 0.008
6.535	6617	0.035	0.981	13.619	14:0 ISO	4.49	ECL deviates -0.000	Reference 0.006
7.044	3013	0.038	0.972	14.000	14:0	2.03	ECL deviates -0.000	Reference 0.006
7.983	46631	0.039	0.960	14.623	15:0 ISO	30.96	ECL deviates -0.000	Reference 0.005
8.119	8302	0.039	0.958	14.713	15:0 ANTEISO	5.50	ECL deviates -0.000	Reference 0.005
8.553	387	0.035	0.954	15 000	15:0	0.26	ECL deviates -0.000	Reference 0.005
9.188	1951	0.040	0.949	15.389	16:1 w7c alcohol	1.28	ECL deviates -0.002	
9.344	4971	0.041	0.948	15.484	Sum In Feature 2	3.26	ECL deviates -0.004	16:1 ISO I/14:0 3OH
9.577	11222	0.046	0.946	15.626	16:0 ISO	7.35	ECL deviates -0.001	Reference 0.003
9.793	685	0.042	0.945	15.758	16:1 w11c	0.45	ECL deviates -0.001	
9.956	15800	0.044	0.944	15.858	Sum In Feature 3	10.32	ECL deviates -0.006	15:0 ISO 2OH/16:1w7c
10.190	6079	0.044	0.943	16.000	16:0	3.97	ECL deviates -0.000	Reference 0.004
10.562	1606	0.045	0.942	16.218	15:0 20H	1.05	ECL deviates -0.001	
10.855	6613	0.045	0.940	16.390	ISO 17:1 w10c	4.30	ECL deviates -0.002	
10.979	7824	0.047	0.940	16.462	ISO 17:1 w5c	5.09	ECL deviates -0.001	
11.118	2078	0.044	0.940	16.543	17:1 ANTEISO A	1.35	ECL deviates -0.003	
11.267	12573	0.043	0.939	16.631	17:0 SIO	8.17	ECL deviates -0.001	Reference 0 005
11.424	2141	0.044	0.939	16.723	17:0 ANTEISO	1.39	ECL deviates -0.000	Reference 0.004
	4971				SUMMED FEATURE 2	3.26	12:0 ALDE ?	unknown 10.928
							16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
	15800				SUMMED FEATURE 3	10.32	16:1 w7c/15 iso 20H	15:0 ISO 2OH/16:w7c

Solvent AR	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
447011437	151017	151017	100.00	144550	12	0.002	0.005

	Similarity Index
TSBA40 (Rev 4.10) Bacillus	0.752
B. cereus	0.752
B.c.GC subgroup A*	0.752
B. thuringiensis canadensis sv.**	0.619
B. thuringiensis kurstakii**	0.523

It is understood for purposes of this disclosure, that various changes and modifications may be made to the invention that are well within the scope of the invention. Numerous other changes may be made which will readily suggest themselves to those skilled in the art and which are encompassed in the spirit of the invention disclosed herein.

The specification contains citations to references such as patents, patent applications, and publications. Each is hereby incorporated by reference for all purposes.